Development of LCMS-Based Fully Validated Bioanalysis of CDR Peptides in Antibody Drugs by Fab-Selective Proteolysis: nSMOL Protocol

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Purpose
Molecular target drugs are largest number in the development in anticancer drugs, and a half of this has become into monoclonal antibody drugs. In the antibody drug development, clinical pharmacokinetics (PK) analysis of each drug is very important for the determination of the individual dose levels or drug efficacy. PK parameters such as plasma concentration of antibodies are often analyzed by conventional ligand binding assays (LBA). The LBA may have some significant limitation, for example the screening of the good anti-idiotypic antibodies for each antibody drug should be indispensable. For the bioanalytical assays, competitive and affinity tests in each biological matrix are necessary. Therefore regulated and robust analytical methods for the direct detection of antibody drugs will be desired in preclinical and clinical applications for drug characterization and PK assessments independent of a variety of antibodies or biological taxonomy sources.

Recently, we have published the novel analytical method for monoclonal antibody quantitation named nano-surface and molecular-orientation limited (nSMOL) proteolysis. This chemistry is LCMS-based antibody complementarity-determining region (CDR) peptide-targeting quantitation through the Fab-selective proteolysis by the limiting protease access to the substrate. Briefly, immunoglobulin G (IgG) with antibody drugs fraction was collected from diluted human plasma and immobilized in Protein A resin pore (size: 100 nm). And proteolysis was performed by immobilized trypsin on the surface of FG nanoparticles (diameter: 200 nm). Owing to these two diameter difference, limited proteolysis on antibody Fab was successful. Using this nSMOL protocols, we have challenged to develop the fully validated bioanalysis conditions for many antibody drugs.

Methods
All validation study was performed in accordance with the Guideline on Bioanalytical Method Validation in Pharmaceutical Development from Notification 0711-1 of the Evaluation and Licensing Division, Pharmaceutical and Food Safety Bureau, the Ministry of Health, Labour and Welfare, dated July 11, 2013 for small molecule LCMS parameters. The structure determination of antibody tryptic peptides and fragments was performed by LC/ion-trap/TOF/MS and Mascot analysis. Identified peptides were assigned by ClustalW multiple sequence alignment, and confirmed the sequence position including CDRs. These CDR-containing peptides could be the candidate for specific signature peptides of each antibody. And next, CDR peptides were analyzed by LC/triple-quadrupole/MS, and determined the optimal conditions of multiple reaction monitoring (MRM) and electrospray ionization interface. The MRM transitions were selected the highest ion as quantitation, and two ions for structure confirmation. Furthermore, selected MRM transitions were verified the interference from plasma matrix. Finally, specific MRM transitions for signature peptides of each antibody drugs were validated bioanalysis according to the guideline criteria such as selectivity, lower limit of quantitation (LLOQ), calibration curve, precision and accuracy, matrix effect, carryover test, dilution integrity, and storage at -20 degC and -80 degC and processed sample stability at 4 degC. LCMS condition are following: solvent A, 0.1% formic acid; B, 0.1% formic acid with acetonitrile; flow rate, 0.4 ml/min; analytical time, 5-7 min; column, Shim-pack GISS C18 (2.1 x 50 mm); interface, 300 degC; DL, 250 degC; heat block, 450 degC; MRM dwell time, 10 msec. P14R synthetic peptide was selected as universal internal standard for all antibodies.

Results
Until today, we have been successful in the full validation development for five antibodies, Trastuzumab, Bevacizumab, Nivolumab, Cetuximab, and Rituximab. The linear quantitation range of these antibodies were approximately from 0.5 to 300 microgram/ml within 20% for LLOQ, and 15% for another concentration settings adequate for the validation guideline criteria and clinical PK data of each antibody drug in the interview form. And now, we are now trying the enhanced sensitivity protocols up to 0.05 microgram/ml order for the microdose assays. And more, nSMOL approach have been even enough for the normal freezing storage at -20 degC. These advantages will be expected to support for the broad and regulated PK. We believe that this nSMOL assay is suitable for routine analysis of plasma antibody concentration to study the correlations between its PK profile and drug efficacy.

Conclusion
nSMOL protocol is focused on the two features: antibody structure-indicated analysis, and complementarity-determining region (CDR)-targeting quantitation. This strategy enabled maintaining antibody specificity while substantially decreasing the massive peptide analytes and residual protease contamination. Therefore practical validation of antibody drug bioanalysis are capable. nSMOL protocol of antibody CDR peptides are made possible by LCMS-based TDM independent of a variety of monoclonal antibody drugs, PK-guided treatment, or the feasibility of the multiplex assays for combination therapy in the near future. We measured the plasma concentration of Bevacizumab or some other antibody drugs in patients with recurrent non-small cell lung cancer according to nSMOL protocols. This approach will be applicable for many new antibody drugs and biosimilar verifications. Furthermore, the clinical PK trials based on LCMS may be expected to aid acceleration of the development of many biopharmaceuticals.